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Abstract: Pancreatic amyloidosis and loss of and cells have been shown to occur in cats with diabetes mellitus, although the number of studies currently available is very limited. Furthermore, it is not known whether pancreatic islet inflammation is a common feature. The aims of the present study were to characterize islet lesions and to investigate whether diabetic cats have inflammation of the pancreatic islets. Samples of pancreas were collected postmortem from 37 diabetic and 20 control cats matched for age, sex, breed, and body weight. Histologic sections were stained with hematoxylin and eosin and Congo red; double labeled for insulin/CD3, insulin/CD20, insulin/myeloperoxidase, insulin/proliferating cell nuclear antigen, and glucagon/Ki67; and single labeled for amylin and Iba1. Mean insulin-positive cross-sectional area was approximately 65% lower in diabetic than control cats (P = .009), while that of amylin and glucagon was similar. Surprisingly, amyloid deposition was similar between groups (P = .408). Proliferation of insulin- and glucagon-positive cells and the number of neutrophils, macrophages, and T (CD3) and B (CD20) lymphocytes in the islets did not differ. The presence of T and B lymphocytes combined tended to be more frequent in diabetic cats (n = 8 of 37; 21.6%) than control cats (n = 1 of 20; 5.0%). The results confirm previous observations that loss of cells but not cells occurs in diabetic cats. Islet amyloidosis was present in diabetic cats but was not greater than in controls. A subset of diabetic cats had lymphocytic infiltration of the islets, which might be associated with -cell loss.

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ENDOCRINE PANCREAS IN CATS WITH DIABETES MELLITUS

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Short title: endocrine pancreas in diabetic cats

Abstract

Pancreatic amyloidosis and loss of α- and β-cells have been shown to occur in cats with diabetes mellitus, although the number of studies currently available is very limited. Furthermore, it is not known whether pancreatic islet inflammation is a common feature. The aims of the present study were to characterize islet lesions and to investigate whether diabetic cats have inflammation of the pancreatic islets. Samples of pancreas were collected postmortem from 37 diabetic and 20 control cats matched for age, gender, breed and body weight. Sections were stained with hematoxylin and eosin, Congo red, double-labelled for insulin/CD3, insulin/CD20, insulin/myeloperoxidase, insulin/PCNA and glucagon/Ki67, single-labelled for amylin and Iba1. Mean insulin-positive cross sectional area was approximately 65% lower in diabetic than control cats (P=0.009) while that of amylin and glucagon was similar. Surprisingly, amyloid deposition was similar between groups (P=0.408). Proliferation of insulin-positive and glucagon-positive cells and the number of neutrophils, macrophages, T (CD3) and B (CD20) lymphocytes in the islets did not differ. The presence of T and B lymphocytes combined tended to be more frequent in diabetic (8/37=21.6%) than control (1/20=5.0%) cats. The results confirm previous observations that loss of β-cells but not of α-cells occurs in diabetic cats. Islet amyloidosis was present in diabetic cats, but was not greater than in controls. A subset of diabetic cats had lymphocytic infiltration of the islets, which might be associated with β-cell loss.

Keywords: Cat, diabetes mellitus, pancreas, islets, amyloid.

Diabetes mellitus (DM) is one of the most common endocrinopathies in cats and its incidence is increasing because of a rise in predisposing factors, such as obesity and physical inactivity.[1,23] Although DM is frequently diagnosed in cats, few studies have characterized the histopathological changes occurring in the endocrine pancreas. In one investigation islet lesions were found in 33 of 35 (94.3%) diabetic cats, including islet cell atrophy and hydropic degeneration (27/35, 77.1%) and moderate to severe amyloidosis (8/35, 22.9%).[5] It was concluded that islet cell lesions were likely associated with DM, but the relevance of islet amyloidosis in the pathogenesis of DM was uncertain because of its low prevalence and lack of association with glycemic control or survival. Other studies have shown that the prevalence of amyloidosis in diabetic cats is much higher, ranging from 65 to 100%,[19,20,27] which suggests that amyloid deposits play a role in the development of DM. However, the results of those studies were based on a limited number of diabetic and control cats and were often compared with those of former studies rather than with well-matched cats within the same study. In humans, islet amyloid is associated with decreased β-cell area and increased β-cell apoptosis, suggesting that its deposition contributes to the reduced β-cell mass that characterizes Type 2 DM.[14] There has been only one study to date that has characterized pancreatic islet cells immunohistochemically in 6 cats with DM.[20] Compared with healthy cats, there was a 50% loss of glucagon- and insulin-positive cells in the diabetic cats; the number of somatostatin-positive cells did not differ between the two groups.

In light of the paucity of histopathological analyses of the endocrine pancreas in cats with DM, the aim of the present investigation was to characterize islet lesions of diabetic cats. A second goal was to determine whether diabetic cats have pathological evidence of islet inflammation compared with a control population of cats well-matched for age, gender, breed and body weight.

Materials and Methods

Cases and controls

Cats that met the following criteria were included in the study: diabetes mellitus was diagnosed based on established clinical and laboratory findings;[24] died spontaneously or were euthanized at the Clinic for Small Animal Internal Medicine, University of Zurich (Switzerland) between 1997 and 2009; a postmortem examination was carried out; and pancreas samples of sufficient size were available. Randomly selected cats with a similar distribution of age, gender, breed and body weight that died spontaneously or were euthanized because of any other disease during the same corresponding period at the same institution were used as controls provided that pancreas samples were available.

Pancreas histopathology

Pancreas samples, approximately 1.5 cm in length, were fixed in 10% neutral buffered formalin, embedded in paraffin and cut to yield serial 3- to 4-µm-thick sections. The sections were stained with hematoxylin and eosin (HE) and evaluated using light microscopy (Olympus BX-40, Milan, Italy). Sections were stained with Congo red for identification of islet amyloid deposits. Details of HE and Congo red stains are provided in Electronic Supplementary Material 1. All samples were randomly selected and therefore standardization of the pancreas section could not be performed.

Pancreas immunohistochemistry

Double-labelling immunohistochemistry was carried out for glucagon and the antigen Ki67 expressed in G1, S, G2 and M phases of the cell cycle, insulin and the T-lymphocyte antigen CD3, insulin and the B-lymphocyte antigen CD20, insulin and the proliferating cell nuclear antigen (PCNA) expressed in G1 and S phases and insulin and the neutrophil antigen myeloperoxidase.[28] Single-labelling immunohistochemistry was carried out for amylin (islet amyloid polypeptide) and for the macrophage antigen ionized calcium-binding adapter molecule 1 (Iba1).[21]

Immunofluorescence was performed for insulin, somatostatin and pancreatic polypeptide. Details of all specificities are provided in Electronic Supplementary Material 2.

Morphology of the endocrine pancreas

Sections stained with HE were evaluated to identify morphologic lesions of the pancreatic islets. The frequency of hydropic degeneration was calculated in 20 islets examined at 400x magnification.

Morphometry of the endocrine pancreas

For morphometric analysis, insulin-, amylin-, glucagon- and amyloid-positive areas relative to the pancreatic area were calculated using ImageJ software (http://rsb.info.nih.gov/ij/) in 10 fields of stained histological sections at 40x magnification. Large vessels, ducts and interlobular tissue were excluded from measurements. In addition, the ratio of amyloid-positive area to the islet area was calculated in cats that had visible islet amyloidosis; 10 islets were evaluated per cat.

Analysis of the number of endocrine, proliferating and inflammatory cells

The number of insulin-, amylin- and glucagon-immunopositive cells in the exocrine pancreas were counted in 10 fields at 400x magnification considering only scattered cells. The number of somatostatin- and pancreatic polypeptide-positive cells was also counted in 10 fields at 400x magnification, both within the islets and in the exocrine pancreas.

To determine the number of proliferating cells, 1000 cells with positive immunostaining for insulin were used to calculate the number of cells with positive double-labelling for insulin and PCNA and 1000 islet cells were used to calculate the number of islet PCNA-positive cells that were insulin-negative; 300 cells with positive immunostaining for glucagon were used to count cells with positive double-labelling for glucagon and Ki67.

To determine the number of inflammatory cells in the endocrine tissue, 1000 islet cells were used to calculate the number of islet CD3-positive lymphocytes, CD20-positive lymphocytes, myeloperoxidase-positive neutrophils and Iba1-positive macrophages. The number of fields and cells examined to complete the above analyses was arbitrarily chosen.

Statistical analysis

To check the process of matching diabetic and control cats, the age, gender, breed and body weight of cats of the 2 groups were tested using Fisher's exact test and the Mann-Whitney test. Differences in frequency of hydropic degeneration in the islets between diabetic and control cats were analyzed using Fisher's exact test. The Mann-Whitney test was used to identify differences between groups for morphometry of insulin-, amylin-, glucagon- and amyloid-positive areas, for counts of insulin-, amylin- and glucagon-positive cells scattered in the exocrine pancreas, for counts of somatostatin- and pancreatic polypeptide-positive cells in the islets and exocrine pancreas and for counts of myeloperoxidase-positive neutrophils and Iba1-positive macrophages in the pancreatic islets. To compare the number of proliferating cells and of CD3- and CD20-positive lymphocytes in pancreatic islets, because their number was zero in many cases, a score was assigned (i.e. present vs. absent) and the difference was analyzed using Fisher's exact test. To further study the course of amyloidosis, possible correlations between the extent of pancreatic islet deposition and age were examined using Spearman's rank correlation coefficient (rho); islets deposits increased with increasing age in healthy cats in an earlier study.[16] Comparison of amylin- or insulin-positive areas in cats with and without islet amyloidosis was achieved with Mann-Whitney test. $P<0.05$ was considered significant. Pancreas sections and images were assessed by two pathologists (LC, FL or SF, FG) in a blinded fashion and inter-observer discrepancies were resolved by consensus.

Results

Cats

The 37 diabetic cats consisted of 26 (70.3%) neutered males and 11 (29.7%) spayed females with a median age of 11 years (range: 2-18 years) and a median body weight of 4.9 kg (range: 2.2-10.0 kg). There were 29 (78.4%) crossbred cats and 8 (21.6%) purebred cats, which included Siamese (n=2), Maine Coon (n=2), Burmese (n=1), Chartreux (n=1), Oriental shorthair (n=1) and Russian Blue (n=1). Concurrent disorders at death were recorded in 21 (56.7%) diabetic cats and included chronic hepatitis, liver lipidosis and severe chronic renal failure in each of 3 cats, chronic enteritis and chronic stomatitis in each of 2 cats and acute enteritis, acute pancreatitis, asthma, hyperthyroidism, idiopathic epilepsy, idiopathic hypercalcemia, megacolon and multicentric lymphoma in one cat each. Diabetic ketoacidosis was identified in 15 (40.5%) cats at the time of death.

The 20 control cats consisted of 14 (70.0%) neutered males and 6 (30.0%) spayed females with a median age of 12 years (range: 2-17 years) and median body weight of 4.3 kg (range: 2.5-9.0 kg). Seventeen (85.0%) were crossbred cats and 3 (15.0%) were purebred cats, which included 2 Chartreux and one British shorthair. Disorders recorded at death were chronic hepatitis in 3 cats, hypertrophic cardiomyopathy, meningioma and severe chronic renal failure in 2 cats each and acute enteritis, colon carcinoma, fibrosarcoma, hyperthyroidism, immune-mediated anemia and thrombocytopenia, liver carcinoma, liver lipidosis, lung carcinoma, multicentric lymphoma, pneumonia and restrictive cardiomyopathy in one cat each. There were no significant differences between diabetic and control cats with respect to age, gender, breed and body weight.

Morphology, morphometry and cell counts of the endocrine pancreas

Examination of HE-stained sections revealed clear differences in the morphology of the islet cells between diabetic and control cats. Islet cells with clear cytoplasm, compatible with hydropic degeneration (P=0.002), were seen in 13 of 37 (35.1%) diabetic cats but not in the control cats. In

diabetic cats with hydropic degeneration, 70.0% of the islets showed this morphologic abnormality (range: 50.0-90.0%). In 4 diabetic cats with more pronounced hydropic degeneration, a large part of the islets appeared devoid of nuclei (Fig. 1, 2). However, the nuclei that were seen appeared morphologically similar in both groups.

In diabetic cats, morphometric analysis of sections stained for insulin showed a severe reduction in the insulin-positive area relative to the pancreatic area. The median insulin-positive area in diabetic cats was approximately 65% smaller than in controls (diabetic cats, median: 0.54% of cross-sectional area, range: 0.01-5.79%; control cats, median: 1.64%, range: 0.71-2.91%; P=0.009) (Fig. 3, 4, 5). Diabetic cats also had approximately 65% lower median number of insulin-immunostained cells scattered in the exocrine tissue than controls (diabetic cats, median: 1.0 per field at 400x magnification, range: 0-6.3; control cats, median: 3.0, range: 0.2-5.2; P<0.001). Insulin-immunostained cells of diabetic cats with hydropic degeneration had insulin distributed as a thin rim around the area of clear cytoplasm (Fig. 3). To determine whether hydropic degeneration influenced the results of insulin morphometry, the analysis was repeated excluding the 13 diabetic cats with islet cells showing clear cytoplasm; the median insulin-positive area in diabetic cats remained significantly smaller than in controls (P=0.003).

The median amylin-positive area in diabetic cats did not differ from that of controls (diabetic cats, median: 0.32% of cross-sectional area, range: 0.02-3.37%; control cats, median: 0.48%, range: 0.03-1.29%; P=0.557). However, based on morphologic assessment, not only islet cells but also amyloid deposits were amylin-immunopositive (Fig. 6). Similar to insulin, the median number of amylin-immunostained cells scattered in the exocrine tissue of diabetic cats was markedly lower than in control cats (diabetic cats, median: 0.1 per field at 400x magnification, range: 0-5.1; control cats, median: 0.6, range: 0-4.1; P=0.005) (Fig. 7, 8, 9).

Morphometric analysis of sections stained for glucagon did not differ between pancreatic sections of diabetic and control cats (diabetic cats, median: 0.19% of cross-sectional area, range: 0.04-0.92%; control cats, median: 0.21%, range: 0.02-0.79%; P=0.282) nor glucagon-immunostained

cells scattered in the exocrine tissue (diabetic cats, median: 0.4 per field at 400x magnification, range: 0.1-1.7; control cats, median: 0.4, range: 0-1.5; P=0.421) (Fig. 10, 11, 12). Furthermore, the median number of somatostatin- and of pancreatic polypeptide-immunostained cells in the islets and in the exocrine pancreas did not differ between diabetic and control cats (data not shown).

Amyloid deposits were observed in pancreatic sections stained with Congo red in 20 of 37 (54.1%) diabetic cats and in 8 of 20 (40.0%) control cats; the frequency of amyloidosis did not differ between groups (P=0.408). In addition, the median amyloid-positive area relative to the islet area did not differ between diabetic and control cats with amyloidosis (diabetic cats, median: 65.0%, range: 40.3-85.1%; control cats, median: 55.1%, range: 43.9-78.9%; P=0.213) (Fig. 13, 14, 15). The extent of deposition of amyloid in pancreatic islets did not correlate with age in the 20 diabetic cats (rho=0.315, P=0.188), but the age range was narrow with 12 cats between the ages of 10 and 13 years. It was interesting to note that there were no amyloid deposits in the islets of the 6 youngest diabetic cats (2-8 years). Similar to diabetic cats, there was no correlation between amyloid and age in the control group. The youngest of the group (2 years) did not have islet amyloid; only one additional cat was below 8 years and showed amyloidosis. Diabetic and control cats with marked islet amyloidosis had insulin- and glucagon-immunostained cells located around the deposits or near capillaries. Those cats had the largest islets of the whole series.

Of note, by grouping all cats together, those presenting with amyloid deposits in the islets had larger median amylin-positive area than those without amyloid deposits (cats with amyloidosis, median: 0.79% of cross-sectional area, range: 0.06-3.37%; cats without amyloidosis, median: 0.30%, range: 0.02-1.13%; P=0.007). Differently, the median insulin-positive area did not differ between cats presenting or not amyloid deposits (cats with amyloidosis, median: 1.15% of cross-sectional area, range: 0.08-5.79%; cats without amyloidosis, median: 0.87%, range: 0.01-2.90%; P=0.226). There was no correlation between amylin- and insulin-positive area in all cats grouped together (rho=0.007, P=0.963).

Proliferation of endocrine islet cells

Proliferating cells double-stained for insulin and PCNA were seen in 5 of 37 (13.5%) diabetic cats and in 1 of 20 (5.0%) control cats (Fig. 16, 17). Their median number and frequency did not differ between groups (P=1.000 and P=0.410, respectively). In addition, proliferating cells positive for PCNA but not for insulin were observed in pancreatic islets of 9 (24.3%) diabetic cats and in only 1 (5.0%) control cat; the frequency tended to be higher in cats with DM (P=0.082) but not the median number (P=0.460). Four of the 5 diabetic cats with proliferating insulin-producing cells also had proliferating cells that were not positive for insulin. The control cat with insulin and PCNA double-labelled cells was not the same cat as the one with proliferating cells not immunostained for insulin. Proliferating cells double-stained for glucagon and Ki67 were observed in only 1 diabetic cat.

Inflammation of the endocrine pancreas

Pancreatic islets of diabetic and control cats had very low numbers of CD3- and/or CD20-positive lymphocytes. In particular, CD3-positive lymphocytes were seen in 3 (8.1%) of 37 diabetic cats (1, 2 and 58 lymphocytes in 1000 islet cells, respectively) but in none of the 20 controls and CD20-positive lymphocytes were observed in 6 (16.2%) diabetic cats (median: 0 in 1000 islet cells, range: 0-67) but in only 1 (5.0%) control cat (1 lymphocyte in 1000 islet cells). Overall, CD3- or CD20-positive lymphocytes were reported in 8 (21.6%) diabetic cats and in 1 (5.0%) control cat, but there was no significant difference between the two groups (P=0.139). Diabetic cats with islet lymphocytes had no more than 2 lymphocytes per 1000 islet cells, except 1 cat that had more than 50. To understand more about the subset of diabetic cats with lymphocytic infiltration of the islets and verify whether an association with early disease onset was present, as described in humans with type 1 DM,[2,4,8] medical records were reviewed and survival from diagnosis was retrieved for 5 of the 8 cases; survival was 2, 2, 5, 48 and 150 days, respectively. Myeloperoxidase-positive neutrophils were common in pancreatic islets of diabetic (median: 4 in 1000 islet cells, range: 0-140) and control cats (median: 3 in 1000 islet cells, range: 0-30) and there was no significant

difference between groups (P=0.412). Iba1-positive macrophages were frequently observed in pancreatic islets of either diabetic (median: 9 in 1000 islet cells, range: 0-230) or control cats (median: 7 in 1000 islet cells, range: 0-180) and there was no significant difference between groups (P=0.247).

Discussion

In the present study, diabetic cats had more frequent hydropic degeneration of islet cells, reduced insulin-positive cross-sectional area of the pancreas, decreased number of insulin- and amylin-positive cells scattered in the exocrine pancreas. Amyloid deposition and amylin-positive cross-sectional area were similar in both groups and there was no clear evidence of more severe inflammation of the pancreatic islets in diabetic cats.

Hydropic degeneration of pancreatic islet cells was seen in more than a third of diabetic cats, but not in controls, confirming previous findings.[5] This morphologic abnormality is nonspecific and suggests that the affected cells are injured by the diabetic milieu or by the accumulation of glucose metabolites generated within the islets.[25] In a previous study, we showed that healthy cats rendered hyperglycemic for 10 days by glucose infusion had hydropic degeneration and accumulation of substantial amounts of glycogen in the islet cells.[28] It is therefore possible that hydropic degeneration seen in the diabetic cats of the present study was also associated with glycogen stored in the cytoplasm. Deposits of glycogen have been described in the islets of diabetic humans and rodents and have been proposed to account, at least in part, for the β-cell secretory defects induced by excess glucose.[17,25] Of note, hydropic degeneration in the diabetic cats of the present study was mostly observed in β-cells and was associated with a thin rim of insulin immunostaining surrounding the clear cytoplasm. Cells with hydropic degeneration that did not display immunoreactivity for insulin, glucagon, somatostatin, or pancreatic polypeptide may represent exhausted β-cells devoid of insulin.

The insulin-positive cross-sectional area and the number of insulin-immunostained cells scattered in the exocrine tissue were markedly lower in diabetic than control cats. These findings were not unexpected and confirm the results of an immunohistochemical study carried out by O'Brien and co-workers,[20] in which 6 diabetic cats had an approximately 50% reduction in insulin-positive cells compared with 6 young healthy cats.

The glucagon-positive cross-sectional area and the number of glucagon-immunostained cells scattered in the exocrine tissue were similar in both groups of cats. These results are in contrast to those of O'Brien and co-workers, in which diabetic cats had decreased numbers of glucagon-immunostained cells compared with young healthy cats.[20] However, that same study reported a similar number of glucagon-immunostained cells in diabetic cats and glucose-intolerant but normoglycemic cats that were matched for age. It is possible that differences in age between diabetic and young cats contributed to the contrasting results of the two studies. In humans with either type 1 or 2 DM and in obese or diabetic mouse and rat models, hyperplasia of α-cells is very common.[6,9] An increase in the number of α-cells has been shown to play a protective role by promoting β-cell proliferation and viability.[6] In a recent study, we showed that at initial diagnosis, cats with DM that later achieved remission had higher plasma glucagon-to-insulin area under the curve ratio compared with cats that remained permanently diabetic.[26] Therefore, α-cells may play a role in protection in the early stages of DM in cats.

The number of somatostatin- and pancreatic polypeptide-positive cells in the islets and in the exocrine pancreas was similar between diabetic and control cats. Similar results have been reported in diabetic cats, in humans with type 2 DM and in some rodent diabetes models, suggesting that δ-cells and PP-cells play a minor role in the pathogenesis of the disease.[13,20,22]

Islet amyloidosis was observed in slightly more than half of the diabetic cats, which is close to the frequency of 65% documented by Yano and co-workers.[27] However, the frequency of islet amyloidosis in cats with DM varies considerably, ranging from 22 to 100%.[5,19,20] Surprisingly, the prevalence and extent of islet amyloidosis did not differ between diabetic and control cats in the present study. The contribution of islet amyloidosis in the development of DM in cats is controversial. In one study, islet amyloidosis was not associated with glycemic control or survival in diabetic cats.[5] Another study found islet amyloidosis in 6 of 7 diabetic cats and in none of 16 controls, which suggested that amyloid deposits are associated with DM.[19] However, in the latter study, controls were young adult cats and the average age of the diabetic cats was 11 years.[19] One

study determined that younger cats have fewer deposits of amyloid,[16] which was in agreement with our results. Yano and co-workers found that the prevalence of islet amyloidosis in diabetic cats and age-matched controls was similar,[27] but the extent of the deposits was greater in cats with DM, suggesting a pathogenic role for amyloid. In the present study, pancreatic islets with marked amyloidosis had insulin- and glucagon-immunostained cells that were often located around the deposits or near capillaries. It is possible that some endocrine cells are lost with increasing amyloid deposition, especially if distant from the vascular supply of nutrients. Based on the above collective findings, we hypothesize that microscopically visible insular amyloid is not the primary cause of DM in cats. Nonetheless, it might contribute to disease progression, which has been postulated in cats and demonstrated in humans with type 2 DM.[10,14,27] It is worth noting that in the present study the analyses were conducted only by means of light microscopy, Congo red and amylin-immunostaining. Recent studies showed that the soluble oligomers of amylin (i.e. precursors of larger conglomerates of insoluble amyloid) are much more toxic to β-cells than the insoluble amyloid or the soluble monomers and fibrils of amylin.[15] Unfortunately the use of Congo red identifies only insoluble amyloid deposits and amylin-immunostaining was unspecific. Indeed, with the latter both insoluble amyloid and islet cell amylin were identified in cats, as demonstrated by direct microscopic assessment of the islets. Non specific binding of antibodies used for amylin to amyloid was previously shown in a model of experimentally induced islet amyloidosis in cats.[11] The lack of specificity of amylin-immunostaining likely explains the increased amylin-positive area observed in cats with islet amyloidosis as compared to those without amyloidosis and the fact that diabetic cats had less amylin-positive cells scattered in the exocrine pancreas than controls but similar amylin positive-area, whereas with insulin-immunostaining diabetic cats had both lower insulin-positive scattered cells and area. Further studies are therefore required to identify soluble oligomers of amylin with a specific immunostaining in cats and investigate their role in the pathogenesis of DM.

The number of T (CD3) and B (CD20) lymphocytes, neutrophils and macrophages in pancreatic islets was similar in both groups. However, although significant differences were not documented, T or B lymphocytes were observed in 21.6% of diabetic cats and in only 5.0% (1 cat) of controls. This observation suggested that infiltration of lymphocytes is involved in the pathogenesis of DM in a subset of affected cats. However, the number of islet lymphocytes was very low in all but one of the cats in our study. To the authors' knowledge, there are no studies on the prevalence of lymphocytic infiltration of islets in diabetic cats. To date, lymphocytic infiltration of pancreatic islets has been reported in only 2 diabetic cats.[7,18] Lymphocytes contribute to the autoimmune destruction of β-cells, which is characteristic of type 1 DM but not type 2 DM in humans.[2] However, lymphocytic infiltration in the islets of type 1 diabetic humans is highly variable; 44-78% of early-onset diabetic patients have islet lymphocytes, whereas none are present 4 months after diagnosis.[2,4,8] Therefore, if the subset of diabetic cats in the present study had insulitis similar to type 1 DM in humans, the low number of lymphocytes documented suggests that a late stage of the inflammatory process was identified in most cases. Survival was known for 5 of the 8 cats with lymphocytic infiltration of the islets and in 4 cases it was below 2 months from diagnosis of DM. However, it should be noted that most diabetic cats are brought to the veterinarian after some weeks to months from onset of clinical signs. It is thus possible that DM developed much earlier in these cats with lymphocytic infiltration. Therefore, the role of time in lymphocytic infiltration of the islets in these cats remains unclear. Whether cats with lymphocytes within the islets have a disorder similar to human type 1 DM remains speculative, especially in light of the fact that circulating autoantibodies against β-cells and insulin have not been identified in the sera of newly diagnosed or treated diabetic cats.[11] Unfortunately, serum was not available for autoantibody testing in the subset of diabetic cats with islet lymphocytes. Studies in humans with type 2 DM and in a rodent model of the disease showed that mild infiltration of pancreatic islets with inflammatory cells can occur, but in contrast to type 1 DM, these cells were macrophages or neutrophils and not lymphocytes.[3,12] In

the present study counts of macrophages and neutrophils were not different between pancreatic islets of diabetic and control cats.

Our study had several limitations. Samples of pancreas were collected postmortem and did not include biopsy specimens obtained during the course of the disease. It is therefore possible that some of the results would have differed if pancreatic biopsies had been obtained at the time of initial diagnosis. In addition, endocrine cells are not evenly distributed in the left and right lobe or body of the pancreas in cats.[20] Because of the retrospective nature of the study, it was not possible to determine whether all samples were derived from the same part of the pancreas. Absence of standardization in preparation of samples might have therefore caused failure to notice variation of islet structure or islet density according to pancreatic location. However, the effect of this potential bias was expected to be minor because the location of sampling was random in both groups of cats.

In conclusion, there was a substantial loss of insulin-immunostained cells in the pancreatic islets of diabetic cats, but no loss of glucagon-, somatostatin-, or pancreatic polypeptide-immunostained cells. The loss of amylin-immonostained cells was evident in the exocrine pancreas but not in the islets due to cross-reactivity of the immunostaining with amyloid. Although islet amyloid was often seen in diabetic cats, its frequency did not differ from that of a group of well-matched control cats. The role of islet amyloid in the development of feline DM is therefore unclear. Insulitis, which is a typical finding in type 1 DM in humans, seems to be uncommon in diabetic cats.

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Declaration of conflicting Interests

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Figure legends

Figures 1-2. Pancreas, cat. HE. **Figure 1.** Islets of a diabetic cat with hydropic degeneration; the asterisk shows segment of an islet devoid of nuclei. **Figure 2.** Pancreatic islet of a control cat.

Figures 3-4. Pancreas, cat. Immunohistochemistry (IHC) for insulin and proliferating cell nuclear antigen (PCNA). **Figure 3.** Intensity of insulin immunostaining (red) and the number of insulin-positive cells are reduced in the islet of a diabetic cat with amyloid deposit and insulin is distributed as a thin perivacuolar rim in cells with hydropic degeneration (arrow). **Figure 4.** Normal insulin immunostaining intensity in the islet with amyloid deposit of a control cat. **Figure 5.** The ratio of insulin-positive area to pancreatic area is significantly smaller in diabetic cats compared to control cats; DM, diabetes mellitus.

Figures 6-8. Pancreas, cat. IHC for amylin. **Figure 6.** Islet of a diabetic cat with amyloid deposit and islet cells immunostained for amylin (brown). **Figure 7.** Amylin-positive cells scattered in the exocrine pancreas of a diabetic cat. **Figure 8.** Amylin-positive cells scattered in the exocrine pancreas of a control cat. **Figure 9.** The number of amylin-positive cells scattered in the exocrine pancreas is significantly smaller in diabetic cats compared to control cats; DM, diabetes mellitus.

Figures 10-11. Pancreas, cat. IHC for glucagon. **Figure 10.** Glucagon immunostaining (red) intensity in the islet of a diabetic cat. **Figure 11.** Control cat shows similar immunostaining intensity. **Figure 12.** The ratio of glucagon-positive area to pancreatic area does not differ between diabetic and control cats (P=0.282); DM, diabetes mellitus.

Figures 13-14. Pancreas, cat. Congo red. **Figure 13.** Diabetic cat with deposition of amorphous, eosinophilic, extracellular material (amyloid) within the islet. **Figure 14.** Similar findings are

observed in a control cat. **Figure 15.** In cats with amyloidosis, the ratio of amyloid-positive area to islet area does not differ between diabetic and control cats (P=0.213); DM, diabetes mellitus.

Figures 16-17. Pancreas, cat. IHC for PCNA and insulin. **Figure 16.** Nuclear immunostaining for PCNA (brown) shows a proliferating β-cell (arrow) in the islet, as demonstrated by concomitant insulin immunolabeling (red) in the cytoplasm. **Figure 17.** Islet cells in a control cat do not show proliferation.